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TITLE: The Structural Basis for the Role of CHK as a Tumor Suppressor Protein in Human Breast Cancer

PRINCIPAL INVESTIGATOR: Jerome E. Groopman, M.D.
Gordon D. Webster, Ph.D.

CONTRACTING ORGANIZATION: Beth Israel Deaconess Medical Center
Boston, Massachusetts 02215-5399

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6. AUTHOR(S)

Jerome E. Groopman, M.D.
Gordon D. Webster, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Beth Israel Deaconess Medical Center
Boston, Massachusetts 02215-5399
E-Mail :jgroopma@caregroup.harvard.edu

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13. ABSTRACT *(Maximum 200 Words)*

The goal of this project is to determine how CHK functions at the molecular level, crystals of the closed and purified, recombinant CHK SH2 domain will be obtained for X-ray diffraction studies from which we can determine its precise, three-dimensional, molecular structure. Our research will focus upon the structural details of how CHK recognizes the tail of the active Her-2/neu receptor.

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Introduction:

We have made considerable progress towards the goals of the project. This work set the stage for developing structural information that may assist in designing novel therapeutics against breast cancer based on mimicking the activity of CHK as an inhibitor of Her2/neu pathway.

Body:

Experiments were conducted as described in the original proposal. In addition, advances were made using different expression systems to obtain purified CHK proteins for structural analysis.

Key Research Accomplishments:

Over the past year, we successfully expressed different truncated variants of SH2 domain of human CHK from the pGEX vector in E. coli. Each of the variants was purified by affinity chromatography and subjected to preliminary crystallization trials using hampton screens. One of the variants yielded preliminary crystals which diffracted to a resolution of 2.5 Å and extensive optimization yielded crystals which diffracted to a resolution of 1.8 Å. The crystal structure of the SH2 domain of CHK was solved at a resolution of 2.5 Å by molecular replacement method and deposited in the data bank (PDB ID: 1JWO).

Unfortunately, attempts to co-crystallize the SH2 domain with several synthetic peptide analogs of HER2/neu receptor did not yield diffractable crystals.

The crystal structure of the SH2 domain was modeled with a peptide using energy minimization. In addition, the kinase domain of CHK was expressed and affinity purified to ~1mg per liter culture from Pichia pastoris. To date, we are unable to express full length and some truncated variants of CHK from Pichia pastoris.

The SH2 domains of CHK and Csk share ~80% sequence homology. Comparison of the structure of SH2 domain of CHK with that of Csk could yield useful information with regards to the interaction with the HER2/neu receptor. To that end, we cloned and expressed from the pGEX vector, in E.coli, the SH2 domains of CHK.

Thus, we have made considerable progress towards our goal but have not yet achieved the ultimate objective of a co-crystal of CHK with the Her2/neu receptor. Our efforts continue using parallel strategies to overcome the pitfalls we face in certain expression systems and feel that these new avenues may soon be fruitful.

Reportable Outcomes: We plan to report the methods for crystallization of CHK SH2 domain and have deposited in the databank the crystal structure of the CHK SH2 domain with a resolution of 2.5 Å.

Conclusions:

CHK is an important regulator of breast cancer growth and spread and appears to act by interaction with the Her2/neu receptor. Our work has advanced to the field in obtaining some structural information on CHK and set the stage for further studies that could lead to novel therapies against breast cancer.

References: none

Appendices: none